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Liquid storage at 4°C of previously frozen red cells

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Fresh human blood was collected in citrate-phosphate-dextrose, frozen by a high-glycerol technique, and stored at −80°C. The red cells were thawed, deglycerolized, and resuspended in a final wash solution, ADSOL (Fenwal Laboratories), or an additive solution (AS) containing glucose, adenine, mannitol, and phosphate. The cells were then stored at 4 to 6°C for 21 days and assayed weekly for adenosine triphosphate and 2,3 diphosphoglycerate, pH, glucose use, and lysis. AS and, to a lesser extent, ADSOL produced metabolic profiles similar to or better than profiles of cells not frozen and stored in commercially available additive solutions. AS offers a potential post-thaw preservative solution for red cells that would greatly increase the flexibility and reduce the expense of using frozen blood. A sterile post-thaw storage capability will make the stockpiling of frozen red cells a practical concept for both military and civilian blood banks- **TRANSFUSION** 1987;27: 496-498.

THE CRYOPRESERVATION of red cells for transfusion was developed 20 years ago and is used routinely in blood banks for the storage of rare blood types or type O cells.[1] The most commonly used procedure, the high-glycerol technique, uses 6 *M* glycerol as a cryopreservative that is approved for storage at −80°C for up to 3 years, although longer storage periods are feasible. Before reinfusion, the cells are thawed and washed with saline by a two-step procedure to remove the glycerol. Washing is done aseptically in one of several approved mechanical "washing machines."[1] Frozen-thawed red cells must be used within 24 hours of processing because the deglycerolizing procedures are not recognized as "closed" systems, and thus sterility is not assured. In addition, the red cells processed with current methods have only minimal nutrient support.

Since the armed services have decided to stockpile frozen red cells, this laboratory and several others are developing methods to deglycerolize cells under sterile conditions. If red cells were sterile after deglycerolization and were given nutrient support, they should be storable at 4°C for 2 to 3 weeks after thawing with at least 75 percent retention of viability and acceptable red cell function. A post-thaw storage capability for red cells would greatly enhance their flexibility as a red cell component, since they could be shipped to hospitals and used like other red cell components.

In this report, we show the in vitro metabolic response of deglycerolized red cells to storage for 21 days at 4°C. The cells were suspended either in: 1) final wash solution; 2) ADSOL (Fenwal Laboratories, Deerfield, IL) or 3) an additive solution (AS) containing glucose, adenine, mannitol, and phosphate.

Materials and Methods

Units of frozen red cells were obtained from the Frank Camp Memorial Blood Center, Fort Knox, Kentucky. The units were frozen within 3 days of collection by the Meryman technique[2,3] and maintained at −80°C for 2.75 years. The units were thawed and deglycerolized in our laboratory using a cell washer (Model 115, Haemonetics Corp., Braintree, MA) with the procedure of Meryman and Hornblower.[2] Four units of red cells were stored in each of three solutions: 1) control cells in a final wash solution containing 200 mg per dl glucose in isotonic saline; 2) ADSOL solution (100 ml; ADSOL containing 2.2 g glucose, 750 mg mannitol, 27 mg adenine, and 900 mg sodium chloride in 100 ml of water); or, 3) AS solution (50 ml unit; containing 1.4 g glucose, 30 mg adenine, 750 mg mannitol, and 1.1 g trisodium phosphate hydrate in 0.45% sodium chloride solution). This latter solution was added to the cells using 0.22-μm filtration and sterile-fill techniques. The red cells scheduled to receive ADSOL were first packed to a hematocrit of 70 percent. Each unit was stored at 4°C and assayed weekly for 3 weeks.

Analysis of red cell adenosine triphosphate (ATP), 2,3-diphosphoglycerate (2,3-DPG), glucose, supernatant hemoglobin, and pH (at 37°C) was done as reported previously.[4] Osmolarity was measured on an osmometer (Model 3D II, Advanced Instruments Inc., Needham, MA). At the end of the storage period, sterility testing was performed on all units. The units were cultured in thioglycolate for both aerobes and anaerobes for a 1-week period.

Statistical analysis of the data was performed using BMDP 2V repeated measures analysis of variance, with multiple comparisons done by Fisher's least significant difference test.[5,6] Alpha (Type I error) for all tests was set at 0.05.

Results

All deglycerolized red cell units had osmolarities and supernatant hemoglobin levels within the acceptable range, below 340 mOsm and 200 mg per dl Hb, respectively.[1] The

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final red cell units had hematocrits near 50 percent and calculated red cell freeze-thaw recoveries between 85 and 95 percent. All units were sterile at the end of the 21-day post-thaw storage period.

The mean red cell ATP and 2,3-DPG levels are shown in Figure 1. There were no significant differences in ATP or 2,3-DPG levels between the control and ADSOL data, except for the 21-day ATP values. The AS cells, when compared with ADSOL or control cells, had significantly higher ATP levels throughout storage and higher 2,3-DPG levels at Days 7 and 14. P50 values, measured on random samples, correlated with 2,3-DPG levels, starting at 23 to 25 torr on Day 0 and dropping to 13 to 14 torr at Day 21.

Measurements of pH of the red cell storage-mixtures measured at 37°C are shown in Figure 2. Because the AS solution pH is 11.2, the AS-treated cells show a mean initial rise in pH from 6.72 to 7.04. Significantly higher pH levels were maintained throughout storage with AS as compared to that with ADSOL or saline-glucose. All units metabolized approximately 100 mg per dl of glucose during 21 days of storage and had at least 100 mg per dl of glucose remaining at 21 days.

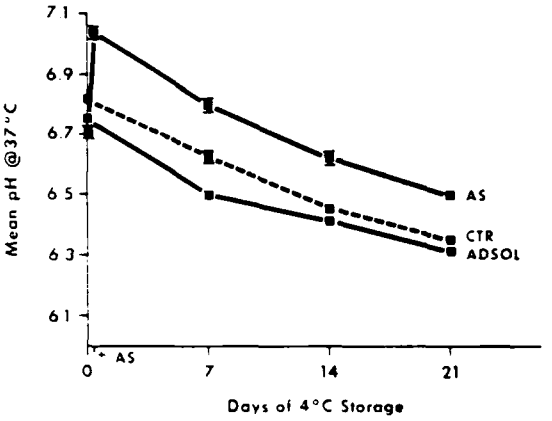

FIG. 2. The pH (mean ± SEM) of red cell suspensions stored at 4°C for 21 days.

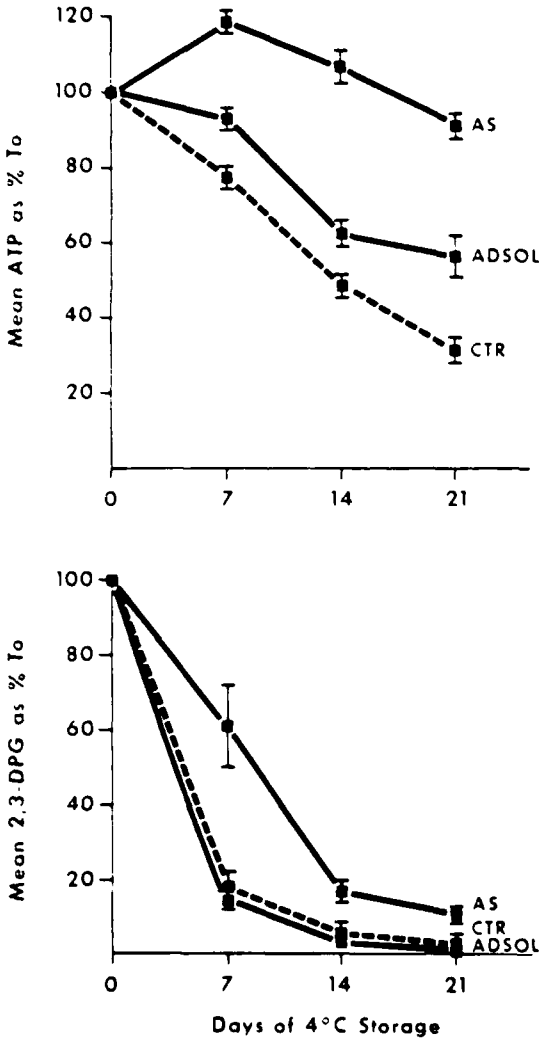

FIG. 1. Mean red cell ATP and 2,3-DPG concentrations during post-thaw storage, expressed as percent of time-zero. Variance is ± SEM. To = time zero.

Supernatant hemaglobin concentrations are shown in Figure 3. Basal values after deglycerolization were 100 mg per dl. Levels increased linearly during the 21-day storage period. Mean red cell lysis on Day 21 was 1.6 percent for AS cells, 7.7 percent for ADSOL cells, and 12 to 20 percent for the control cells, which showed much larger variation.

Discussion

If stockpiles of frozen red cells are to have practical value in times of war or in mass casualties, there must be some flexibility in the distribution and use of the thawed cells. This flexibility is attained best by assur-

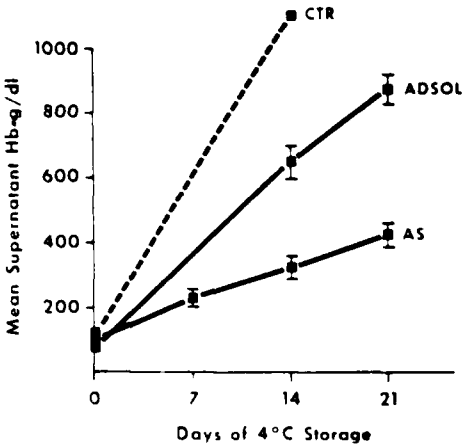

FIG. 3. Supernatant hemoglobin (mean ± SEM) during 21 days of post-thaw storage.

ing that the freeze-thaw-deglycerolization process is sterile and that the cells have the necessary nutrient support for subsequent refrigerator storage. A consensus among military blood bankers is that a 4°C storage period of 14 to 21 days after thawing would be desirable (Hohn DE, personal communication, US Army Health Services Command, Fort Sam Houston, TX, June 1986).

The in vitro data for ATP, pH, and glucose use suggest that AS and, to a lesser extent, ADSOL are capable of maintaining red cell metabolism for 21 days of storage at 4°C. If these data on post-thaw storage are compared with similar data sets for liquid preservation using citrate-phosphate-dextrose-adenine (CPDA-1 or CPDA-2), then, based on the red cell survival data for CPDA-1,2, deglycerolized red cells stored in AS or ADSOL should have 24-hour survivals above the currently acceptable Food and Drug Administration guideline of 75 percent.[7,8] Red cell survival studies will be done to confirm the efficacy of AS and ADSOL for post-thaw preservation.

The superior maintenance of 2,3-DPG in the AS group is probably due to the elevated pH obtained by mixing the red cells with the pH 11 AS solution. The use of tribasic sodium phosphate in the AS raises the initial pH of the red cell suspension to that seen for blood freshly drawn into CPD, whereas the ADSOL solution does not modify the deglycerolized red cell pH. AS also appears to be more effective than ADSOL in retarding red cell lysis. The reason for this is not clear, since the solutions contain the same amount of mannitol. The effect may be related to the ability of AS to increase suspension pH and to the slight hyperosmolarity of the AS (460 mOsm).

A post-thaw preservation period of 3 weeks, which maintained sterile red cells at a quality level equal to or greater than that of CPDA-1, would greatly expand the usefulness and increase the economy of frozen blood. Such cells could be processed at cryopreservation centers and shipped to user hospitals within a several-hundred-mile radius. The hospitals could then bank the cells and use them with the same flexibility as fresh blood. The economy of freezing would improve, since the thawed cells would not be committed to a single surgical patient but, if not used, could be returned to the blood bank and redesignated for another patient. This flexibility is necessary for the practical use of frozen blood in military operations and could prove a practical answer to civilian blood banking during times of emergency or periodic blood shortages.

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